

STUDIES ON THE USE OF CARBON DIOXIDE DISSOLVED IN REFRIGERATED BRINE FOR THE PRESERVATION OF WHOLE FISH

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ABSTRACT

Although storing fish in refrigerated seawater has many advantages over storing them in ice, the use of refrigerated seawater also has several disadvantages, one of which is the difficulty in controlling the growth of spoilage bacteria in the fish. Reported here is the effect on the growth of bacteria in rockfish and chum salmon of dissolving carbon dioxide in brine. Storing the fish in the refrigerated brine treated with carbon dioxide inhibited the growth of the bacteria, retarded the rate at which the fish decrease in quality, and increased their storage life by at least 1 week.

Refrigerated seawater as a medium for cooling, storing, and transporting fish has many advantages, which have been well documented (Idyll, Higman, and Siebenaler, 1952; Osterhaug, 1957; Cohen and Peters, 1962; Peters and Dassow, 1965; Roach et al., 1967).

This medium, however, also has disadvantages. These include the excessive uptake of water by species of low oil content, such as sole and cod, and an increase in total salt. Controlling the growth of spoilage bacteria in fish stored in refrigerated seawater also presents a problem (Roach et al., 1967). This problem results from the blood, dissolved protein, and visceral contents accumulated in the seawater during the storage of the fish. For these reasons, fish held in refrigerated seawater are not necessarily of better quality than are those held for the same period in ice. Nor can fish necessarily be held longer in refrigerated seawater than in ice before spoilage occurs.

This laboratory recently began a study of methods for increasing the effectiveness of refrigerated seawater as a medium for preserving fish. The investigation is timely because fishermen are finding it increasingly difficult to locate catches on traditional fishing grounds. This reduced abundance requires longer stays at sea, which sometimes result in the landing of fish of less than optimum quality.

Use of carbon dioxide gas dissolved in refrigerated seawater seemed promising as an inhibitor of the spoilage bacteria. Stansby and Griffiths (1935), for example, found that whole haddock and haddock fillets stored in an atmosphere of carbon dioxide kept almost twice as long as did those stored in air. Castell (1953) demonstrated that carbon dioxide showed promise of being a useful preservative for salted fish held in 12% brine. Carbon dioxide has been used effectively to extend the storage life of refrigerated meat and poultry products (Wheaton, 1960) and is known to have bacterial inhibiting properties (King and Nagel, 1967). Fiskeriministeriets Forsøgslaboratorium (1968) noted that, in limited experiments on holding fish in tanks, carbon dioxide decreased the rate at which their quality was degraded. Wayne I. Tretsven (1968, personal communication) showed that the shelf life of fresh silver salmon refrigerated in a mixed atmosphere of carbon dioxide, oxygen, and nitrogen was significantly extended beyond that of fresh silver salmon refrigerated in air.

Rockfish is normally iced aboard the fishing vessel and may be held for as long as 7 to 10 days before being landed. Chum salmon is frequently held in refrigerated brine aboard cannery tenders and may be held aboard the vessel for as long as 7 days. With both methods of holding, the quality of the fish may be poor if they must be held for longer periods. This

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study is specifically concerned with the effects that holding in modified refrigerated brine containing dissolved carbon dioxide³ has on the storage life and quality of rockfish and chum salmon.

PRINCIPLES OF THE MODIFIED REFRIGERATED BRINE SYSTEM

EFFECTS OF DISSOLVING CO₂ IN REFRIGERATED BRINE

Carbon dioxide is a relatively inert chemical compound. It is almost odorless and, in the gaseous form, is colorless. Combined with water, it forms carbonic acid, a weak acid. Depending on the conditions, only part of the CO₂ added to the water is dissolved. The undissolved CO₂ either rises to the surface of the solution and is wasted away or else becomes suspended as gas bubbles, thereby forming carbonated water. The amount of CO₂ that can be dissolved by water depends on the pressure and temperature. The higher the pressure of the CO₂ and the lower the temperature of the water (at least, down to 35° F), the greater the amount of CO₂ dissolved. (We found that lowering the temperature below 35° F did not increase solubility.)

When chilled brine is saturated with CO₂, its pH is reduced from about 7.5 or higher to about 4.0. This change in pH from the alkaline to the acid condition helps to inhibit the growth of bacteria that contribute to spoilage (Wheaton, 1960). But pH control is not the only operative factor. Dissolved CO₂ seems to inhibit the metabolic processes of spoilage organisms and, of course, temperature control is important in slowing growth rate.

Although the modified refrigerated brine technique produces positive effects with regard to the control of bacteria, the addition of CO₂ can, under certain conditions, produce undesirable side effects. These effects are manifested in the form of accelerated corrosion rates of metals exposed to seawater containing high concentrations of the dissolved CO₂.

³ In this report, the term "modified refrigerated brine" will henceforth mean brine containing dissolved carbon dioxide (CO₂).

REFRIGERATED BRINE EQUIPMENT

The equipment we used consisted of two fiber glass-insulated 55-gal epoxy-coated drums and a brine chiller.

We cooled the drums by circulating refrigerated brine from the brine chiller through 200 ft of 3/4-inch polyvinyl chloride tubing wound around the outside of the drums in series and returned to the chiller (Figure 1).

Polyethylene liners with a capacity of about 30 gal were suspended in the drums by clamps. (The purpose of the liners was to keep the fish away from the cold sides of the drum, where they tend to freeze.)

Each drum was equipped with a Moyno^o pump (Figure 2) for recirculating chilled brine (a solution containing 3.3% sodium chloride).⁴ The brine was circulated by the pumps through a fitting in the bottom of the polyethylene liner. It was then forced through fish that had been placed in the liners, whereupon it overflowed back into the drums. The brine in the drums was picked up by a suction hose and recycled through the pumps at the rate of 10 gal/min. For maximum diffusion into the brine, the CO₂ gas was fed into the suction side of the circulating pump at the rate of 0.2 ft³/hr. The brine in the other drum was left untreated for use as a control.

STORAGE LIFE AND QUALITY OF ROCKFISH HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All measurements reported here were made in duplicate.

³ The use of trade names is merely to simplify descriptions; no endorsement is implied.

⁴ Sodium chloride brine was used in lieu of natural seawater because clean seawater was not convenient to the laboratory. However, this technique has previously been used by Collins (1950), Davis and Clark (1944), and others and found to give good results. In comparative experiments conducted by Roach and Harrison (1954) and more recently by this laboratory (unpublished), the test results showed that fish held in refrigerated brine were of equal quality to fish held in refrigerated seawater.



FIGURE 1.—Arrangement of the brine chiller (on the left); the pump is (on the floor) for circulating the chilled brine through the cooling coils shown wrapped around the uninsulated holding tanks.

Bacteriological Measurements

Materials and methods.—Described here are the rockfish and brine samples we used and the methods of making total plate counts.

The rockfish, *Sebastes flavidus*, were caught in a trawl off the coast of Oregon. In the preparation of the samples, 130 lb. of the fresh, whole fish was divided into two equal lots. Each lot was placed in a polyethylene-lined drum of brine at a one-to-one ratio by weight of fish to brine. At this time, the iced fish had been out of the water 24 hr. The ratio by weight of fish-to-brine was maintained throughout the experiment by removing a known weight of brine at each sampling period.

One tank of brine was treated with CO₂ gas

before the fish were loaded into it. The brine in both tanks was cooled to $31^{\circ} \pm 0.5^{\circ}$ F during the experiment.

Periodically three fish and a sample of brine were removed from each of the storage drums for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made on the fish by the methods described by Pelroy and Eklund (1966). Briefly, the method was as follows: a slice of flesh was removed from near the dorsal side of each fish just posterior to the nape. Each subsequent experimental sampling was made from the same side and area of each fish tested. Forty-five grams of fish from the excised samples was homogenized aseptically



FIGURE 2.—Arrangement of the Moyno pump for recirculating chilled brine in the holding tank (on the left) and the CO₂ cylinder (in the back) and attached CO₂ flow meter (being adjusted by the worker).

with 180 ml of sterile 0.1% peptone solution at 38° F. Serial dilutions in 0.1% peptone-water were prepared for pour plates from the homogenate. Total plate counts were made by use of a TPY medium (0.5% yeast extract, 1.5% trypticase, 0.5% phytone, 0.2% glucose, 0.5% NaCl, and 1.5% agar). Counts were made on the brine by taking 1-ml samples, making appropriate serial dilutions in the 0.1% peptone-water mixture, and plating out onto the TPY medium. The plates were incubated at 22° C for 5 days.

Results and discussion.—Table 1 gives the results of the total-plate-count analyses on the brines and on the flesh of the rockfish. The data from the untreated brine show that a lag in bacterial growth occurred during the first 3

days of the storage test. After the third day, however, the population of bacteria in the brine increased rapidly.

Total plate counts made on the brine treated with CO₂ did not show a significant increase in the number of bacteria during the 17 days of storage.

Bacterial growth in the flesh of the rockfish held in the untreated brine was not inhibited during storage. On the 10th day, the fish were judged, by appearance and odor, to be inedible and unfit for testing. At this time, the total plate counts each exceeded a million organisms per gram of flesh. (A total plate count of a million organisms per gram usually indicates flesh of poor quality.)

TABLE 1.—Chemical and microbiological changes occurring in CO₂-treated refrigerated brine and in untreated refrigerated brine, and in the flesh of rockfish held in these brines.

Time in storage	Data on refrigerated brine with added CO ₂							Data on refrigerated brine without CO ₂				
	CO ₂ conc.		pH		Salt conc.	Total bacterial plate count		pH		Salt conc.	Total bacterial plate count	
	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine	Flesh	Brine	Flesh	Flesh	Brine
<i>Days</i>	<i>ppm</i>	<i>ppm</i>			<i>%</i>	<i>no./g</i>	<i>no./ml</i>			<i>%</i>	<i>no./g</i>	<i>no./ml</i>
0	119	1,000	6.7	4.0	0.2	1.2×10 ⁴	1.3×10 ⁴	6.7	6.8	0.2	1.2×10 ⁴	1.3×10 ⁴
3	562	2,332	5.8	5.3	0.5	--	1.6×10 ⁴	6.4	6.9	0.6	7.7×10 ⁴	1.2×10 ⁴
8	842	1,848	6.4	6.0	1.1	1.6×10 ⁴	--	6.5	7.3	1.0	2.4×10 ⁹	2.8×10 ⁸
10	--	--	--	6.0	1.3	--	--	--	--	--	Spoiled ¹	>10 ⁶
14	597	1,452	6.5	6.0	--	1.4×10 ⁴	5.3×10 ⁵	--	--	--	--	--
17	--	--	6.4	6.1	1.8	2.0×10 ⁴	3.8×10 ⁴	--	--	--	--	--

¹ Samples judged by appearance and odor to be inedible and unfit for tasting.

The storage of rockfish in the refrigerated brine containing the CO₂ was terminated after 17 days. At this time, the results of the total plate counts made on the flesh of the fish showed that the microbial population had not changed significantly from the initial total plate count of 10⁴ organisms per gram of flesh.

Chemical Measurements

pH.—The pH of the flesh of the rockfish and of the brines was measured by means of a Beckman combination electrode. The pH of the flesh was measured by inserting the tip of the electrode into the flesh (Patashnik, 1966).

Table 1 gives the results of the pH measurements of the fish and the brine. After 8 days of continuous recirculation, the pH of the control brine changed from a slightly acid condition (pH 6.8) to a slightly alkaline condition (pH 7.3). This change coincided with an increase in the microbial population in the control brine and was probably due to the formation of ammonia and amines from the bacterial degradation of proteins dissolved in the brine.

The initial pH of the brine treated with CO₂ shows the effect of the dissolved CO₂. The measurement was made before the fish were loaded into the brine. The subsequent increase in the pH of the brine in the presence of additional CO₂ may be attributed to the buffering by the soluble proteins in the blood and slime. After the 8th day of the experiment, the pH of the brine treated with CO₂ did not increase significantly.

Between the initial examination and that on

the 3rd day, the pH of the flesh of the fish held in the brine treated with CO₂ dropped appreciably. This change was coupled with an increase in the concentration of the CO₂ in the flesh. As storage continued, the pH returned to the same level (6.4 to 6.5) as that of the flesh held in the untreated brine.

CO₂ concentration.—The concentration of CO₂ in the flesh and brine was measured by the method of Umbreit, Burris, and Stauffer (1957). The procedure was essentially as follows: A slice of fish was removed from the thickest part (dorsal side) of the fish. The sample was then carefully sectioned into horizontal cuts about ¼-inch thick and the individual cuts analyzed. A sample of the flesh or of brine was blended in Tris buffer (hydroxymethyl) aminoethane at a pH of about 9. Five grams of the mixture was added to a Warburg flask, and 0.7 ml of 0.5 M citrate buffer at pH 4.0 was added to the side arm of the flask. After the flask and its contents came to equilibrium at 38° F in a water bath, the contents of the side arm were tipped into the flask. The increase in manometric pressure was recorded at irregular intervals of time ranging up to 10 min. The amount of CO₂ evolved was calculated from a standard curve prepared by determining the changes in pressure after measured amounts of acid were tipped into known concentrations of bicarbonate.

Penetration studies carried out on whole rockfish showed that CO₂ diffused into the flesh very slowly. The maximum depth of penetration into the flesh was 0.75 inch. This depth was reached in about 8 days of storage. The highest

concentration of CO₂ in the flesh (842 ppm) was reached at this time.

The retention of CO₂ in the flesh was given consideration as a potential problem in contributing to an abnormal head-space pressure in canned salmon and to the separation of breading on breaded rockfish products. The initial indications were, however, that the retention of CO₂ will not be a problem. As was remarked earlier, CO₂ is not absorbed well at above-normal storage temperatures. CO₂ will therefore likely be dissipated from the flesh during routine cleaning, heading, and washing operations, which are done at temperatures considerably higher than those of storage. In an experiment in which red salmon were held in modified refrigerated natural seawater and commercially canned, no problems were encountered as the result of CO₂ retention. In canned products such as tuna and shrimp, the retention of CO₂ should not be a problem, because these products are exposed to relatively high preprocessing temperatures.

Additional consideration of these potential problems, however, will be given to the retention of CO₂ in future studies on modified refrigerated brine.

Salt concentration.—The concentration of sodium chloride was measured by the method described by Greig and Seagran (1965). In brief, a plastic-strip indicator containing a sensitized capillary element was placed in a filtered extract of fish and distilled water. After the reading was taken by means of the indicator, the concentration of salt in the extract was read from a standard curve supplied by the manufacturer of the device.

During the first 8 days of storage, the uptake of salt was similar in the fish held in treated brine to that in the fish held in the control brine. Concentrations of salt in the fish held in the untreated brine for longer than 8 days were not determined, because these fish spoiled at about that time. The fish held in the treated brine were analyzed for concentration of salt on the 10th and 17th days of storage. They showed somewhat more uptake of salt at each of these times.

SUBJECTIVE MEASUREMENTS

Raw Rockfish

At each sampling, a trained taste panel determined the effect of storage of the fish in two kinds of brine water. The fish were also evaluated in the round for general appearance and odor.

During the first 3 to 4 days of storage, fish held in either of the two brines were of good color, odor, and texture. By the 5th day, odors occurred in the fish held in the untreated brine. Between the 7th and 10th days, the fish were judged, by appearance and odor, to be unfit for tasting. During this time, the untreated brine had a strong odor of putrefaction and was dark brown.

The fish held in brine treated with CO₂ retained good color, odor, and texture for 17 days. The brine was almost colorless and odorless at the end of the experiment.

Cooked Rockfish

Cooked rockfish were prepared for taste-panel evaluation by the method of Miyauchi, Stoll, and Dassow (1964). The samples of cooked fish were evaluated for appearance, odor, flavor, texture, and overall quality, using a 10-point numerical scale.

Table 2 gives the sensory scores for the cooked samples. The data show that the fish in the untreated brine spoiled between the 7th and 10th days of storage. Except for an increase in saltiness, which occurred in the fish in either

TABLE 2.—Sensory evaluations on the cooked flesh of rockfish held in CO₂-treated refrigerated brine and untreated brine (control).

Time in storage	Overall sensory score ¹		Comments	
	Brine and CO ₂	Brine (control)	Brine and CO ₂	Brine (control)
<i>days</i>				
0	9.0	9.0	--	--
3	8.0	8.0	Slight salty taste	Slight salty taste
7	7.0	6.0	Unobjectionable salty taste	Off-odors
10	--	Spoiled	Unobjectionable firm texture	Foul off-odors
14	7.0	--	Firm texture	--
17	7.0	--	Objectionable salty taste; texture, color, and odor good	--

¹ A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.

of the two storage environments, the fish held in the modified brine water were organoleptically acceptable and of good quality after 17 days of storage. The subsequent refrigerated shelf life of this product was not determined.

STORAGE LIFE AND QUALITY OF CHUM SALMON HELD IN MODIFIED REFRIGERATED BRINE

OBJECTIVE MEASUREMENTS

Both bacteriological and chemical measurements were made. All the reported measurements were made in duplicate.

Bacteriological Measurements

Materials and methods.—Described here are the samples of salmon and of brine and the methods of making total plate counts.

About 300 lb. of fresh seine-caught chum salmon, *Oncorhynchus keta*, were obtained in the round from Bellingham, Wash. The salmon, which weighed about 10 to 13 lb. each, were divided into two lots of equal size. Each lot was loaded at a one-to-one brine-to-product ratio by weight into a drum of circulating brine containing 3.3% NaCl (see footnote 4). The salmon had been held in ice and, at this time, were less than 24 hr out of the water.

The brine was precooled and treated as was described in the section on rockfish.

Three salmon and a sample of brine were removed periodically for examination. The fish samples were used to make both the objective and subjective measurements at each sampling.

Total bacterial plate counts were made of the

bacteria on the skin of the fish. Samples of the bacteria were obtained by the swab technique of Tretsven (1963). Briefly, the procedure consisted in swabbing the skin of the fish with a sterile swab through a 2 cm² hole cut into the center of a sterilized metal template. The tip of the swab was broken off in such a way that it fell into 10 ml of a 0.1% peptone solution, which was then mixed. Appropriate serial dilutions were made from this mixture and were plated out on the TPY medium (see the bacteriological section described under rockfish) for the determination of total bacterial counts.

In previous experiments at this laboratory, the swab technique gave results similar to those obtained from samples of flesh. Because of this finding and because of the relative simplicity of the swab technique, we used it in this experiment. Total plate counts of the bacteria in the brine were made by the method used in the rockfish experiment.

Results and discussion.—Table 3 shows the total plate counts made on the untreated and treated brines. In the control brine, the bacterial population steadily increased during the 18-day experiment. About the 7th day of storage, the brine evidenced a slight odor of spoilage. By the 11th day, the untreated brine smelled intensely putrid. At that time, the total plate count exceeded 10⁹ organisms per milliliter.

The effect of CO₂ is demonstrated by the essentially unchanged bacterial population in the treated brine during the experiment. The bacterial population increased between the 3rd and 9th days but appeared then to stabilize. During the experiment, the brine remained odorless and,

TABLE 3.—Chemical and microbiological changes occurring in CO₂-treated refrigerated brine and in untreated refrigerated brine, and on the flesh of salmon held in these brines.

Time in storage	Data on refrigerated brine with added CO ₂				Data on refrigerated brine without CO ₂			
	pH of brine	Salt conc.	Total bacterial plate count		pH of brine	Salt conc.	Total bacterial plate count	
			Skin	Brine			Skin	Brine
<i>days</i>		%	<i>no./cm</i>	<i>no./ml</i>		%	<i>no./cm</i>	<i>no./ml</i>
0	4.0	0.3	1.1×10 ⁶	1.3×10 ⁴	7.1	0.3	1.1×10 ⁶	1.3×10 ⁴
3	5.5	0.6	7.7×10 ⁴	9.3×10 ⁴	6.8	0.6	--	8.1×10 ⁴
9	--	1.3	1.9×10 ⁶	1.4×10 ⁶	--	1.2	2.4×10 ⁶	2.6×10 ⁶
11	5.5	--	3.2×10 ⁴	2.1×10 ⁶	6.8	--	1.0×10 ⁶	5.0×10 ⁶
18	5.5	1.3	3.8×10 ⁴	2.0×10 ⁴	6.8	1.4	3.3×10 ⁶	3.5×10 ⁷

except for a small amount of suspended protein, remained clear and colorless.

The number of bacteria on the skin of the salmon held in the untreated brine increased more than 20 fold. After the salmon had been in storage for 9 days, the number of bacteria on the skin increased from its original value of 1.1×10^5 to 2.4×10^6 /cm². At 11 days of storage, the salmon were judged, on the basis of odor, to be spoiled.

Swab tests on the skin of the salmon held in the brine treated with CO₂ showed that essentially no growth of bacteria occurred during the 17 days of storage.

Chemical Measurements

pH.—The pH of the brine was measured as was described earlier. The pH of the flesh was not measured. Table 3 shows the pH values for the brines.

Except for the initial value of 7.1, the untreated brine had a pH of 6.8 throughout the experiment. As yet, we do not know if the difference in pH of the brines used for holding rockfish and salmon is related to a difference in the spoilage patterns of the two species.

The pH of the brine treated with CO₂ remained in a stable acid condition throughout the experiment.

CO₂ concentration.—No analyses were made for CO₂ in the flesh or in the brine. However CO₂ was continuously metered into the experimental brine at the same rate as that in the experiment with rockfish.

Salt concentration.—For greater accuracy than is possible with the simple rapid method of analysis described earlier, the concentration of NaCl in the flesh was measured by the Volhard method (Horwitz, 1960). The sample analyzed was taken from both fillets of a single fish. The fillets were mechanically comminuted and thoroughly mixed before the sample was taken, and the analyses were made in duplicate.

As was true with the rockfish, treating the brine with CO₂ had no effect on the rate of salt

uptake. Salmon held in both brines showed progressive and similar increases in concentration of salt to a maximum of 1.3% to 1.4% in the flesh at 9 days.

SUBJECTIVE MEASUREMENTS

Raw Salmon

At each sampling, the salmon were examined in the same manner as had been the rockfish.

At the beginning of the experiment, the untreated salmon had a bright appearance and a thick covering of colorless slime. After 4 to 5 days, however, they had lost their natural brightness. They remained slimy, but the slime had begun to turn yellow. By the 11th day, the salmon looked blanched and smelled spoiled. At this time, the brine was dark brown and had an intense odor of spoilage.

The salmon held in the brine treated with CO₂ retained most of their natural color during the experiment. By the end of the first week of storage, however, only a trace of slime remained on their skins. On the 18th day, when the experiment was terminated, the salmon still had a good appearance and were free of off odors. The brine was almost colorless and almost odorless.

Cooked Salmon

The taste-test scores (Table 4) show that the samples from both storage environments were equally acceptable through the first 7 days of storage.

TABLE 4.—Sensory evaluations on the flesh of chum salmon held in CO₂-treated refrigerated brine and in untreated refrigerated brine.

Time in storage	Overall sensory score ¹		Comments	
	Brine and CO ₂	Brine (control)	Brine and CO ₂	Brine (control)
<i>days</i>				
0	9.0	9.0	--	--
3	8.0	8.0	--	Slight salty taste
7	8.0	8.0	--	--
11	7.0	Spoiled	Unobjectionable salty taste; firm texture	Odor of uncooked flesh putrid
18	6.0		Color, odor, and texture good; objectionable salty taste	--

¹ A score of 10 denotes a product of highest quality; one of 5 denotes a product of borderline quality.

By the 11th day, however, the taste panel rated those held in the untreated brine as being unacceptable.

In contrast, the salmon held 18 days in the treated brine were acceptable. The panel judged that these salmon had good texture and color but that they had only fair flavor. The deterioration in the flavor may have been due in part to the presence of absorbed salt (a salt concentration of about 1.0% is generally considered to be optimum) but was due mostly to chemical changes that occurred in the flesh during storage.

SUMMARY AND CONCLUSIONS

The purpose of the work reported here was to determine the effect that holding rockfish or chum salmon in refrigerated brine treated with CO₂ would have on their storage life and quality.

Storing rockfish in brine treated with CO₂ increased their storage life by at least 1 week. The CO₂ inhibited bacterial growth and retarded the rate at which the rockfish decreased in quality.

Storing chum salmon in brine treated with CO₂ gave similar results.

This study indicates that the addition of CO₂ to refrigerated brine considerably improves the preservation properties of this medium with respect to bacterial spoilage. The absorption, however, of water, uptake of salt, loss of soluble protein, and the as-yet-undetermined subsequent refrigerated shelf life of the landed product are problems that remain to be solved. At this time, we therefore cannot recommend that rockfish and chum salmon be held in modified refrigerated brine beyond presently accepted storage periods—that is, 8 to 10 days for either species.

Although we do not at present recommend extending the holding times, the reader may wish to keep in mind that the quality of a landed product held in refrigerated brine is significantly improved by the addition of CO₂.

Future modified brine studies will be directed at solving the above mentioned problems and the problems concerned with accelerated corrosion.

LITERATURE CITED

- CASTELL, C. H.
1953. Cool storage of lightly salted fish in kench and under pickle. Fish. Res. Bd. Can., Progr. Rep. Atl. Coast Sta. 56: 17-22.
- COLLINS, J.
1960. Processing and quality studies of shrimp held in refrigerated sea water and ice. Part 1—Preliminary observations on machine-peeling characteristics and product quality. Commer. Fish. Rev. 22(3): 1-5.
- COHEN, E. H., AND J. A. PETERS.
1962. Storage of fish in refrigerated sea water. 1. Quality changes in ocean perch as determined by organoleptic and chemical analyses. U.S. Fish Wildl. Serv., Fish Ind. Res. 2(1): 41-47.
- DAVIS, H. C., AND G. H. CLARK.
1944. Holding sardines in chilled brine. Pac. Fisherman 42(9): 43.
- FISKERIMINISTERIETS FORSØGSLABORATORIUM.
1968. Main experimental results: fresh fish. In Fiskeriministeriets Forsøgslaboratorium, Copenhagen, Denmark, Arsberetning fra Fiskeriministeriets Forsøgslaboratorium for 1967, p. 44-45.
- GREIG, R. A., AND H. L. SEAGRAN.
1965. Technical note no. 1—A rapid field method for determining the salt concentration in fresh and smoked chub. Commer. Fish. Rev. 27(12): 18-21.
- HORWITZ, W. (editor).
1960. Official methods of analysis, 9th ed. Association of Official Agricultural Chemists, Washington, D.C. 832 p.
- IDYLL, C. P., J. B. HIGMAN, AND J. B. SIEBENALER.
1952. Experiments on the holding of fresh shrimp in refrigerated sea water. Fla. State Bd. Conserv., ML 2738, 23 p. (Processed.)
- KING, A. D., JR., AND C. W. NAGEL.
1967. Growth inhibition of a *Pseudomonas* by carbon dioxide. J. Food Sci. 32: 575-579.
- MIYAUCHI, D., N. STOLL, AND J. DASSOW.
1964. Storage life and acceptability studies on the radiation-pasteurized crab meat and flounder. In Application of radiation-pasteurization processes to Pacific crab and flounder, final summary for the period November 1963 thru October 1964, p. 6-31. U.S. At. Energy Comm. Res. Develop. Rep. TID-21404.
- OSTERHAUG, K. L.
1957. Refrigerated sea water bibliography. U.S. Fish Wildl. Serv., Technol. Lab., Seattle, Wash. B-SSU-No. 2. 21 p. (Processed.)
- PATASHNIK, M.
1966. New approaches to quality changes in fresh chilled halibut. Commer. Fish. Rev. 28(1): 1-7.

- PELROY, G. A., AND M. W. EKLUND.
1966. Changes in the microflora of vacuum-packaged, irradiated petrale sole (*Eopsetta jordani*) filets stored at 0.5 C. Appl. Microbiol. 14: 921-927.
- PETERS, J. A., AND J. A. DASSOW.
1965. Improved methods of handling fresh fish in the United States. Part III. - Use of refrigerated sea water. Indo-Pac. Fish. Council, Proc. 11th Sess. Sect. 3: 254-263.
- ROACH, S. W., AND J. S. M. HARRISON.
1954. Use of chilled sea water and brines in place of ice for holding shrimp aboard a fishing vessel. Fish. Res. Bd. Can., Progr. Rep. Pac. Coast Sta. 98: 23-24.
- ROACH, S. W., J. S. M. HARRISON, AND H. L. A. TARR.
1961. Storage and transport of fish in refrigerated sea water. Fish. Res. Bd. Can., Bull. 126, 61 p.
- ROACH, S. W., H. L. A. TARR, N. TOMLINSON, AND J. S. M. HARRISON.
1967. Chilling and freezing salmon and tuna in refrigerated sea water. Fish. Res. Bd. Can., Bull. 160, 40 p.
- STANSBY, M. E., AND F. P. GRIFFITH.
1935. Carbon dioxide in handling fresh fish - had-dock. Ind. Eng. Chem. 27: 1452-1458.
- TRETSVEN, W. I.
1963. Bacteriological survey of filleting processes in the Pacific Northwest. II. Swab technique for bacteriological sampling. J. Milk Food Technol. 26: 383-388.
- UMBREIT, W. W., R. H. BURRIS, AND J. F. STAUFFER.
1957. Manometric techniques. Revised ed. Burgess Publishing Co., Minneapolis, 338 p.
- WHEATON, R. B.
1960. Effects of carbon dioxide on microorganisms with reference to application in controlled atmosphere storage of refrigerated food. Whirlpool Res. Lab. St. Louis, Mo., Proj. 2054, Res. Rep. 1, 34 p. (Processed.)